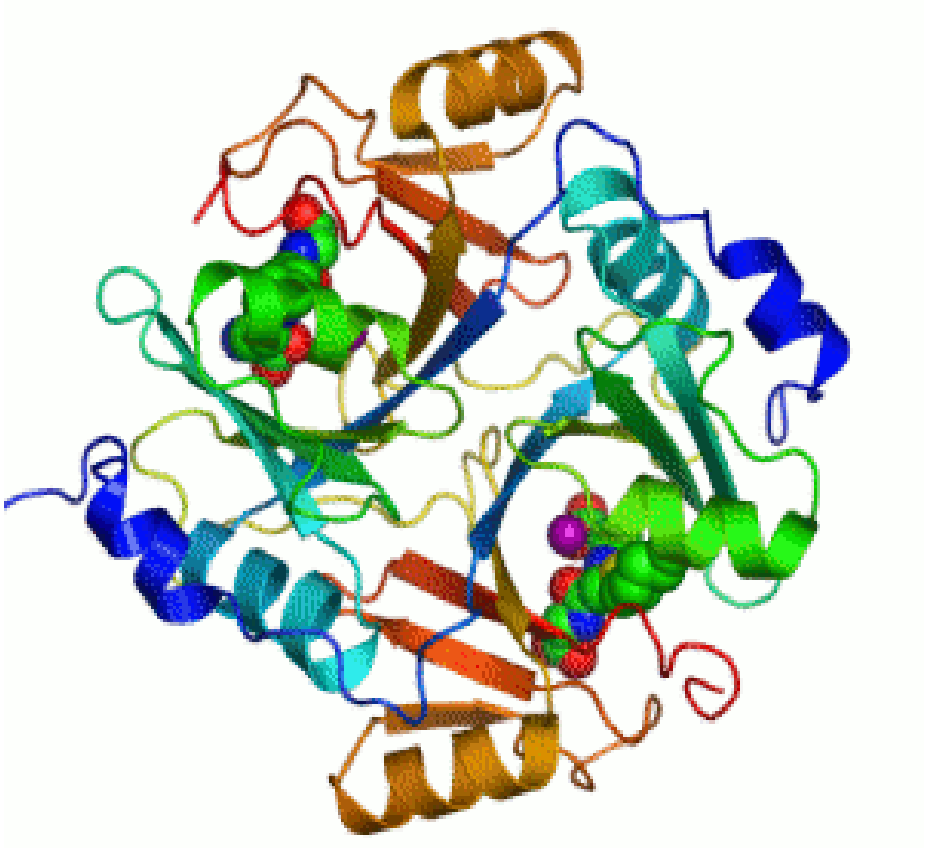
AL-RASHEED UNIVERSITY COLLEGE
DEPARTMENT OF MEDICAL LABORATORY TECHNIQUES

Enzymes

Lecture 4



Prepared By
Dr. Kutaiba I. Alzand & Dr. Rusul H. Hamza

الوحدة الأولى - المحاضرة الرابعة - الزمن: 90 دقيقة

أهداف المحاضرة الرابعة:

يتوقع في نهاية الجلسة أن يكون الطالب قادراً على:

By the end of the lecture, the student should be able to:

1. State the way in which each type of inhibition affects enzyme kinetics and illustrate how each of the three types affects the enzymatic reaction rate using a Lineweaver-Burk plot.
2. List the physiological factors that affect blood enzyme levels.
3. Compare the methods available for analysis of clinically significant enzymes and describe how the rate of an enzyme-catalyzed reaction relates to the amount of enzyme activity present in a system.

موضوعات المحاضرة الرابعة:

➢ REGULATION OF ENZYME ACTIVITY

- Allosteric enzymes
  - Homotropic effectors
  - Heterotropic effectors:
- Covalent modification
- Enzyme synthesis

➢ ENZYMES IN CLINICAL DIAGNOSIS

- Plasma enzyme levels in disease states
- Plasma enzymes as diagnostic tools
- Isoenzymes and heart disease
  - Isoenzyme quaternary structure
  - Diagnosis of myocardial infarction

VIII. ENZYME REGULATION

- The regulation of the reaction velocity of enzymes is essential if an organism is to coordinate its numerous metabolic processes.
- The rates of most enzymes are responsive to changes in substrate concentration, because the intracellular level of many substrates is in the range of the Km.
- Thus, an increase in substrate concentration prompts an increase in reaction rate, which tends to return the concentration of substrate toward normal.
- In addition, some enzymes with specialized regulatory functions respond to allosteric effectors and/or covalent modification or they show altered rates of enzyme synthesis (or degradation) when physiologic conditions are changed.

A. Allosteric enzymes

- Allosteric enzymes are regulated by molecules called effectors that bind noncovalently at a site other than the active site.
- These enzymes are almost always composed of multiple subunits, and the regulatory (allosteric) site that binds the effector is distinct from the substrate-binding site and may be located on a subunit that is not itself catalytic.
- Effectors that inhibit enzyme activity are termed negative effectors, whereas those that increase enzyme activity are called positive effectors.
- Positive and negative effectors can affect the affinity of the enzyme for its substrate ($K_{0.5}$), modify the maximal catalytic activity of the enzyme (V_{max}), or both (Fig. 5.15).
- [Note: Allosteric enzymes frequently catalyze the committed step, often the rate limiting step, early in a pathway.]

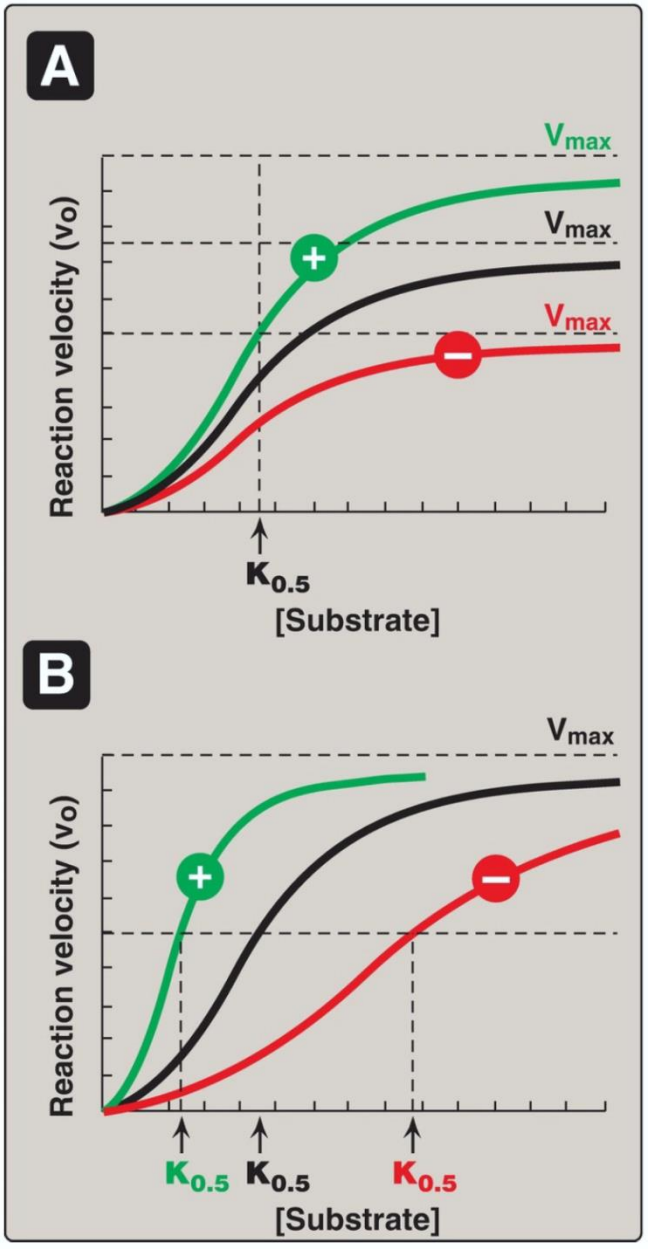


Figure 1.15 Effects of negative **(-)** or positive **(+)** effectors on an allosteric enzyme. A. V_{max} is altered. B. The substrate concentration that gives half-maximal velocity ($K_{0.5}$) is altered.

1. Homotropic effectors:

- When the substrate itself serves as an effector, the effect is said to be homotropic.
- Most often, an allosteric substrate functions as a positive effector. In such a case, the presence of a substrate molecule at one site on the enzyme enhances the catalytic properties of the other substrate-binding sites. That is, their binding sites exhibit cooperativity.
- These enzymes show a sigmoidal curve when vo is plotted against substrate concentration, as shown in Figure 5.15. This contrasts with the hyperbolic curve characteristic of enzymes following Michaelis-Menten kinetics, as previously discussed.

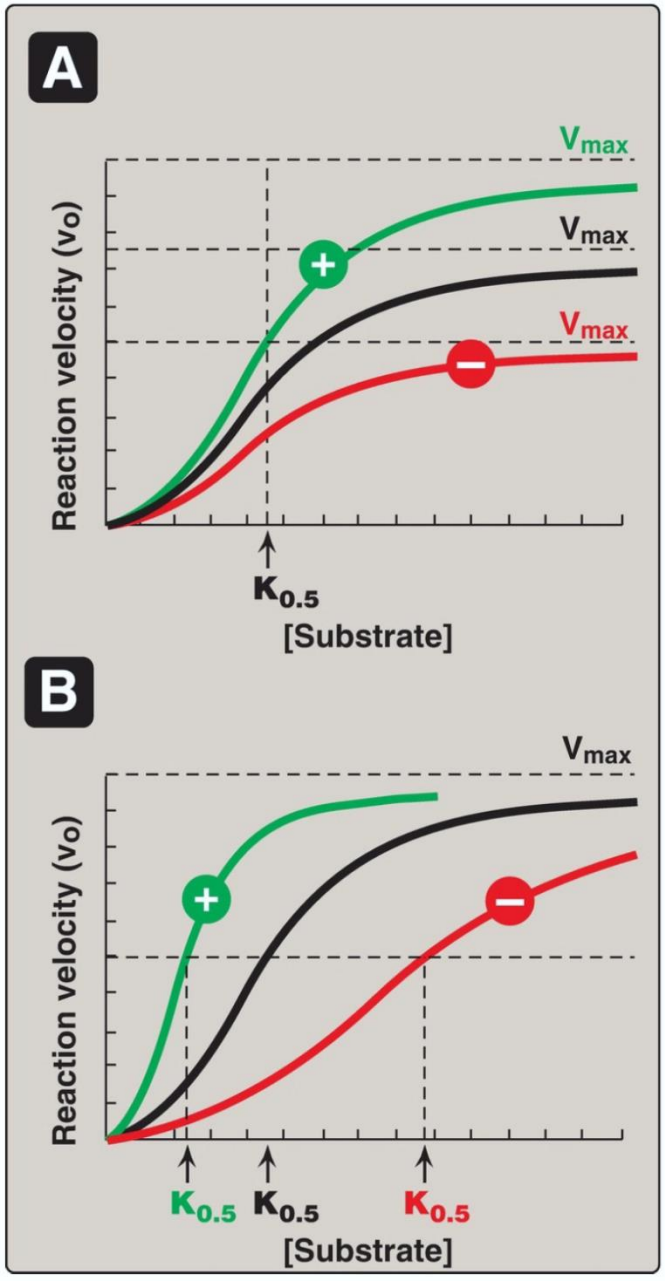


Figure 1.15 Effects of negative **(-)** or positive **(+)** effectors on an allosteric enzyme. A. V_{max} is altered. B. The substrate concentration that gives half-maximal velocity ($K_{0.5}$) is altered.

2. Heterotropic effectors:

- The effector may be different from the substrate, in which case the effect is said to be heterotropic.
- For example, consider the feedback inhibition shown in Figure 5.17. The enzyme that converts D to E has an allosteric site that binds the end product, G.
- If the concentration of G increases (for example, because it is not used as rapidly as it is synthesized), the first irreversible step unique to the pathway is typically inhibited.
- Feedback inhibition provides the cell with appropriate amounts of a product it needs by regulating the flow of substrate molecules through the pathway that synthesizes that product.

- Heterotropic effectors are commonly encountered. For example, the glycolytic enzyme ***phosphofructokinase-1*** is allosterically inhibited by citrate, which is not a substrate for the enzyme.

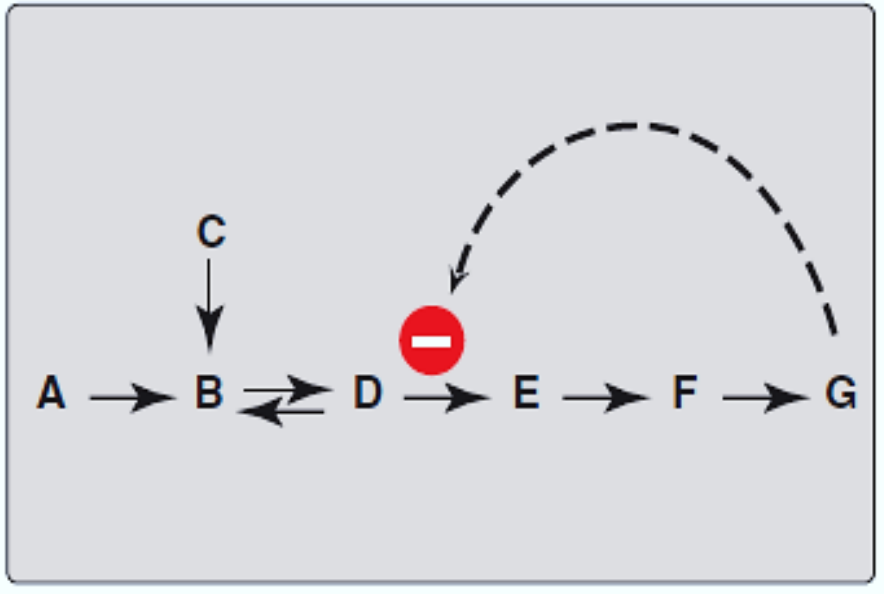
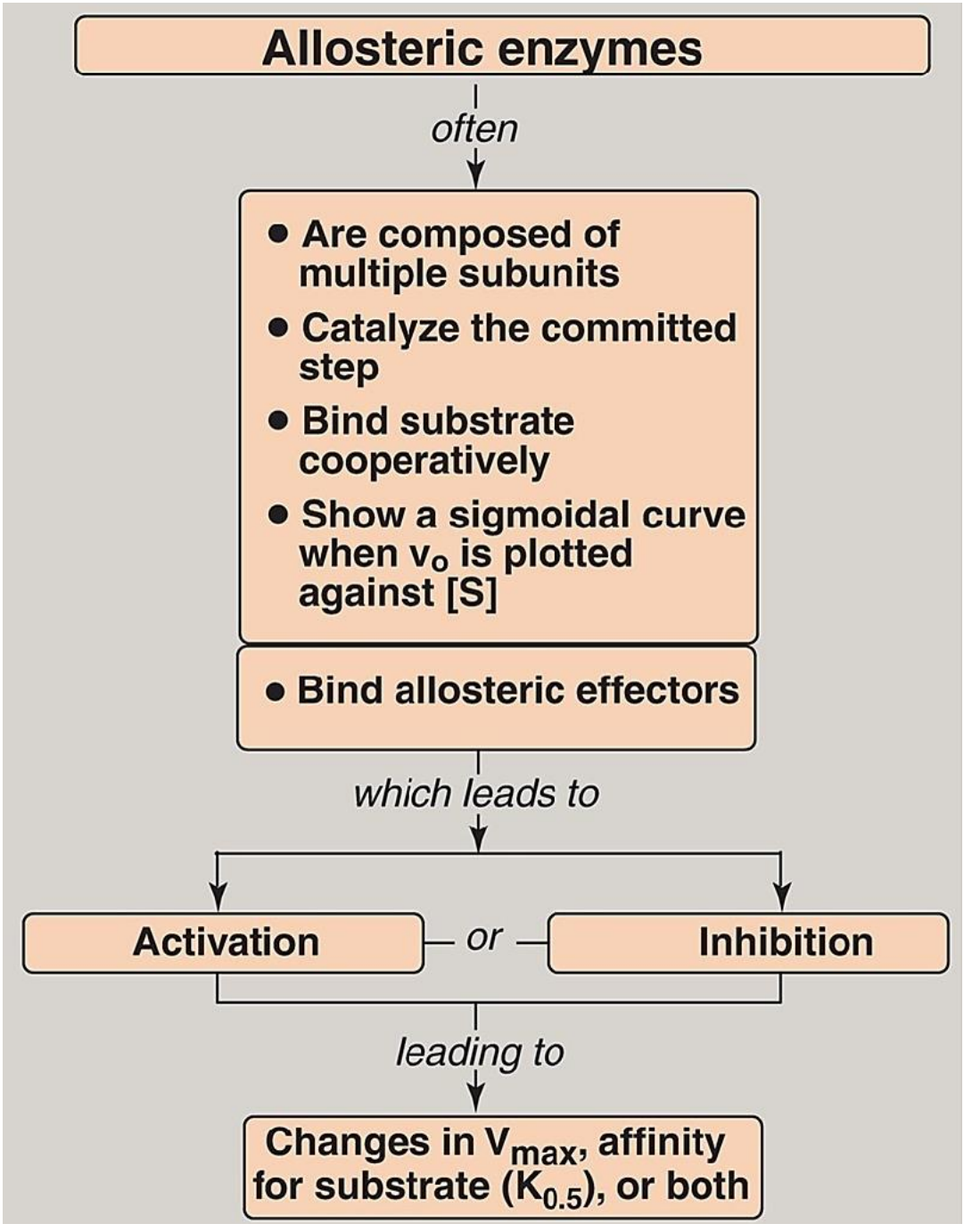


Figure 1.16 Feedback inhibition of a metabolic pathway.


Allosteric enzymes
often
• Are composed of multiple subunits
• Catalyze the committed step
• Bind substrate cooperatively
• Show a sigmoidal curve when v_o is plotted against [S]
• Bind allosteric effectors
which leads to
Activation
or
Inhibition
leading to
Changes in V_{max}, affinity for substrate ($K_{0.5}$), or both

B. Covalent modification

- Many enzymes are regulated by covalent modification, most often by the addition or removal of phosphate groups from specific serine, threonine, or tyrosine residues of the enzyme. Protein phosphorylation is recognized as one of the primary ways in which cellular processes are regulated.

1. Phosphorylation and dephosphorylation: Phosphorylation reactions are catalyzed by a family of enzymes called *protein kinases* that use ATP as the phosphate donor. Phosphate groups are cleaved from phosphorylated enzymes by the action of *phosphoprotein phosphatases* (Fig. 5.17).

2. Enzyme response to phosphorylation: Depending on the specific enzyme, the phosphorylated form may be more or less active than the unphosphorylated enzyme. For example, hormone-mediated phosphorylation of *glycogen phosphorylase* (an enzyme that degrades glycogen) increases activity, whereas phosphorylation of *glycogen synthase* (an enzyme that synthesizes glycogen) decreases activity.

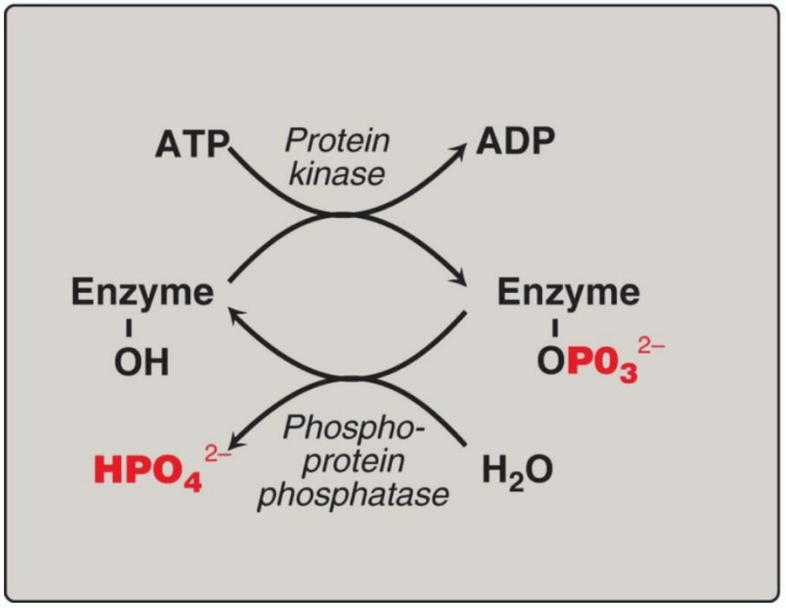


Figure 1.17 Covalent modification by the addition and removal of phosphate groups. [Note: HPO_4^{2-} may be represented as Pi and PO_3^{2-} as P .] ADP = adenosine diphosphate.

C. Enzyme synthesis

- The regulatory mechanisms described above modify the activity of existing enzyme molecules.
- However, cells can also regulate the amount of enzyme present by altering the rate of enzyme degradation or, more typically, the rate of enzyme synthesis.
- The increase (induction) or decrease (repression) of enzyme synthesis leads to an alteration in the total population of active sites.
- Enzymes subject to regulation of synthesis are often those that are needed at only one stage of development or under selected physiologic conditions.
- For example, elevated levels of insulin as a result of high blood glucose levels cause an increase in the synthesis of key enzymes involved in glucose metabolism.
- In contrast, enzymes that are in constant use are usually not regulated by altering the rate of enzyme synthesis.
- Alterations in enzyme levels as a result of induction or repression of protein synthesis are slow (hours to days), compared with allosterically or covalently regulated changes in enzyme activity, which occur in seconds to minutes.

IX. ENZYMES IN CLINICAL DIAGNOSIS

- Plasma enzymes can be classified into two major groups.
- First, a relatively small group of enzymes are actively secreted into the blood by certain cell types. For example, the liver secretes zymogens (inactive precursors) of the enzymes involved in blood coagulation.
- Second, a large number of enzyme species are released from cells during normal cell turnover. These enzymes almost always function intracellularly and have no physiologic use in the plasma.
- In healthy individuals, the levels of these enzymes are fairly constant and represent a steady state in which the rate of release from damaged cells into the plasma is balanced by an equal rate of removal from the plasma. Increased plasma levels of these enzymes may indicate tissue damage (Fig. 5.20).

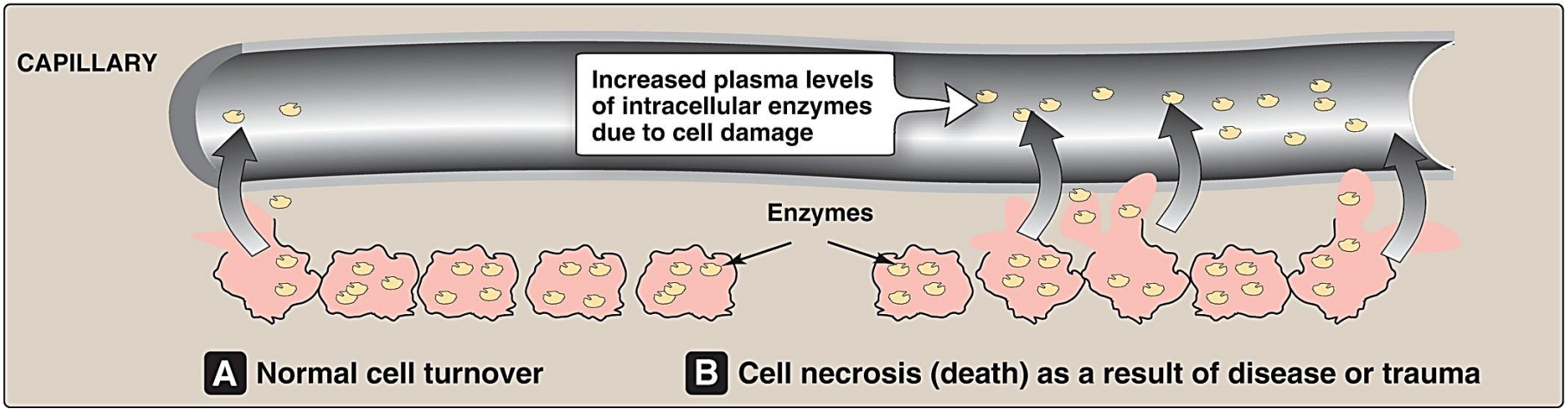


Figure 5.20 Release of enzymes from normal (A) and diseased or traumatized (B) cells.

A. Plasma enzyme levels in disease states

- Many diseases that cause tissue damage result in an increased release of intracellular enzymes into the plasma. The activities of many of these enzymes are routinely determined for diagnostic purposes in diseases of the heart, liver, skeletal muscle, and other tissues. The level of specific enzyme activity in the plasma frequently correlates with the extent of tissue damage. Therefore, determining the degree of elevation of a particular enzyme activity in the plasma is often useful in evaluating the prognosis for the patient.

B. Plasma enzymes as diagnostic tools

- Some enzymes show relatively high activity in only one or a few tissues. Therefore, the presence of increased levels of these enzymes in plasma reflects damage to the corresponding tissue. For example, the enzyme ***alanine aminotransferase*** (***ALT***) is abundant in the liver. The appearance of elevated levels of ***ALT*** in plasma signals possible damage to hepatic tissue. [Note: Measurement of ***ALT*** is part of the liver function test panel.] Increases in plasma levels of enzymes with a wide tissue distribution provide a less specific indication of the site of cellular injury and limits their diagnostic value.

C. Isoenzymes and heart disease

- Isoenzymes (also called isozymes) are enzymes that catalyze the same reaction. However, they do not necessarily have the same physical properties because of genetically determined differences in amino acid sequence. For this reason, isoenzymes may contain different numbers of charged amino acids, which allows electrophoresis (the movement of charged particles in an electric field) to separate them (Fig. 5.21).

- Different organs commonly contain characteristic proportions of different isoenzymes. The pattern of isoenzymes found in the plasma may, therefore, serve as a means of identifying the site of tissue damage. For example, the plasma levels of ***creatine kinase*** (***CK***) are commonly determined in the diagnosis of myocardial infarction (MI). They are particularly useful when the electrocardiogram (ECG) is difficult to interpret such as when there have been previous episodes of heart disease.

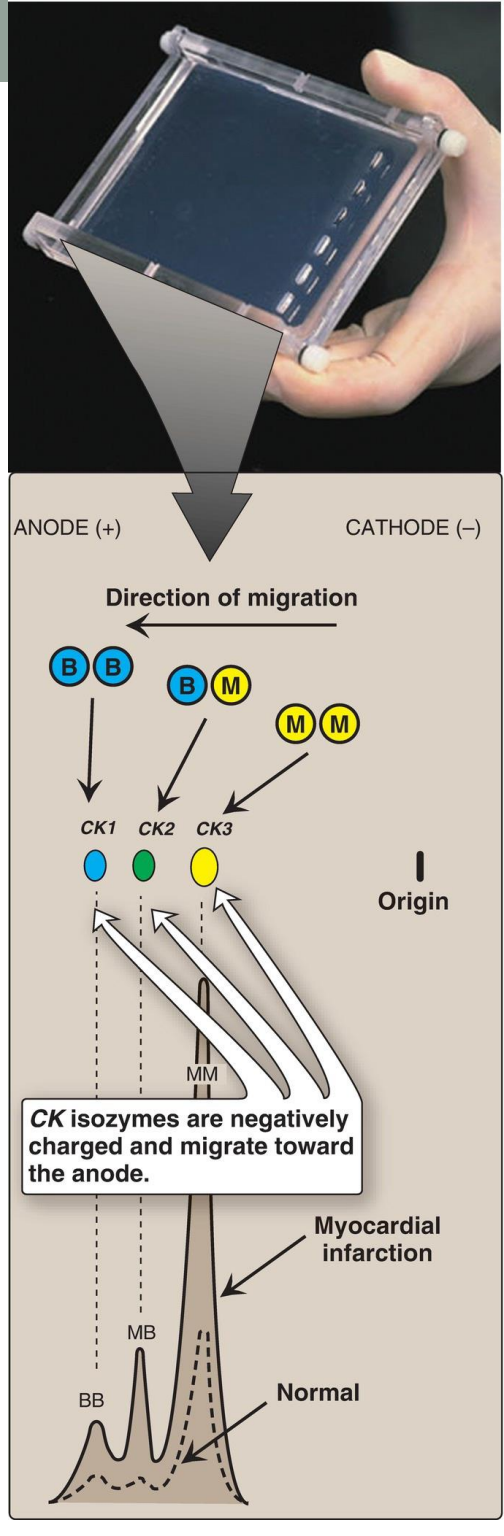
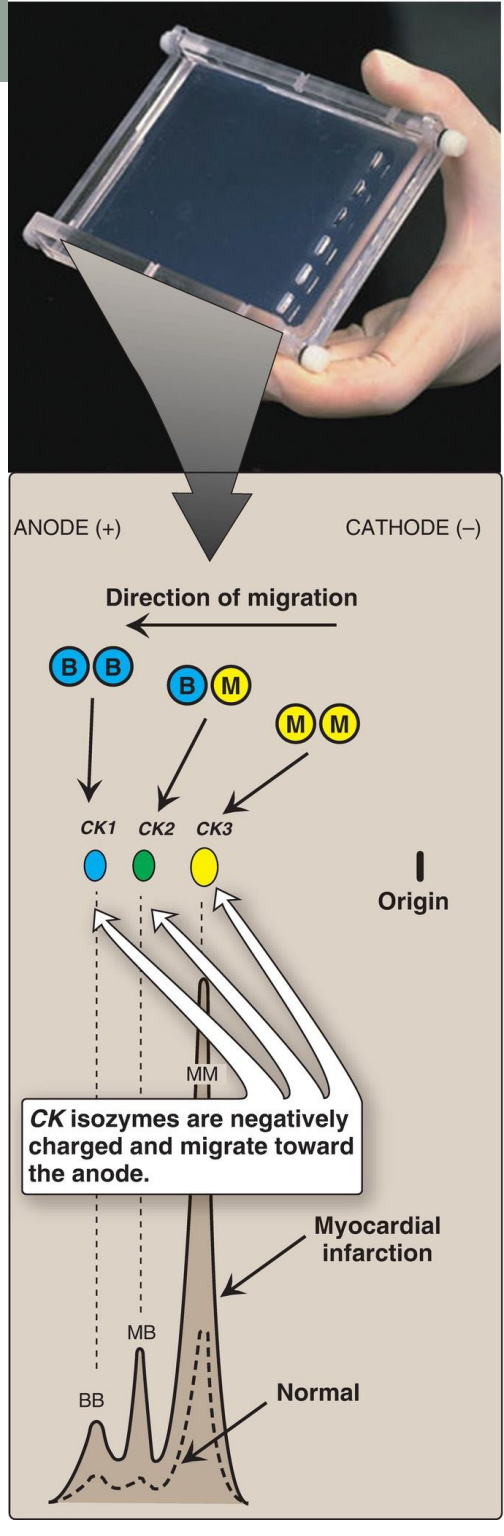


Figure 5.21 Subunit composition, electrophoretic mobility, and enzyme activity of *creatine kinase* (*CK*) isoenzymes.

1. Isoenzyme quaternary structure: Many isoenzymes contain different subunits in various combinations. For example, ***CK*** occurs as three isoenzymes. Each isoenzyme is a dimer composed of two polypeptides (called B and M subunits) associated in one of three combinations: ***CK1*** = BB, ***CK2*** = MB, and ***CK3*** = MM. Each ***CK*** isoenzyme shows a characteristic electrophoretic mobility (see Fig. 5.21). [Note: Virtually all ***CK*** in the brain is the BB isoform, whereas it is MM in skeletal muscle. In cardiac muscle, about one third is MB with the rest as MM.].

2. Diagnosis of myocardial infarction: Measurement of blood levels of proteins with cardiac specificity (biomarkers) is used in the diagnosis of MI. Myocardial muscle is the only tissue that contains >5% of the total ***CK*** activity as the ***CK2*** (MB) isoenzyme. Appearance of this hybrid isoenzyme in plasma is virtually specific for infarction of the myocardium.



- Following an acute MI, ***CK2*** appears in plasma within 4–8 hours following onset of chest pain, reaches a peak of activity at ~24 hours, and returns to baseline after 48–72 hours (Fig. 5.22).
- Troponins T (TnT) and I (TnI) are regulatory proteins involved in muscle contractility.
- Cardiac-specific isoforms (cTn) are released into the plasma in response to cardiac damage. They are highly sensitive and specific for damage to cardiac tissue. cTn appear in plasma within 4–6 hours after an MI, peak in 24–36 hours, and remain elevated for 3–10 days.
- Elevated cTn, in combination with the clinical presentation and characteristic changes in the ECG, are currently considered the "gold standard" in the diagnosis of an MI.

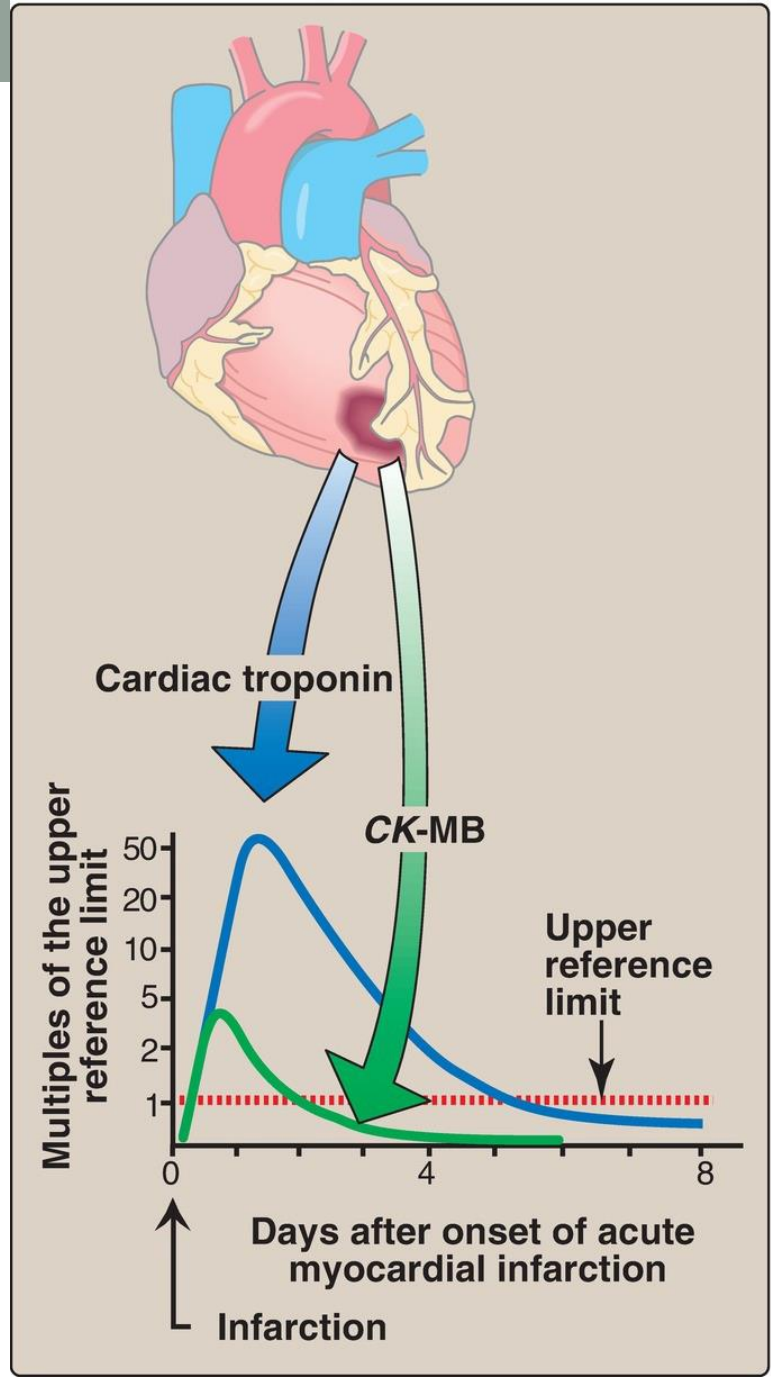


Figure 5.22 Appearance of *creatine kinase* isozyme *CK*-MB and cardiac troponin in plasma after an myocardial infarction. [Note: Either cardiac troponin T or I may be measured.]